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Characterization of polyphenol oxidase from the fruit fly *Bactrocera tau* (Walker) (Diptera: Tephritidae)

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Abstract: 【Objective】 This study aims to determine the activities of polyphenoloxidase (PPO) in the fruit fly *Bactrocera tau* (Walker) during various developmental stages. 【Methods】 The PPO activity and kinetic properties in the 1st, 2nd and 3rd instar larva, pupa and adult were determined with spectrophotomethrical method using catechol as the substrate. 【Results】 The PPO activities varied significantly during different developmental stages of *B. tau*. The enzyme activity in the 3rd instar larva was the highest (434.42 U/mg) and that in pupa was the lowest (231.05 U/mg). At pH 6.5, the enzyme activities in the 1st, 2nd and 3rd instar larva, pupa and adult were 265.42, 358.34, 444.42, 210.02 and 373.99 U/mg, respectively. However, PPO activities decreased dramatically at pH levels above 7.0 or below 5.0. At 34℃ and 37℃, PPO activities stayed at a high level, while the enzyme activities significantly decreased at above 40℃ or below 27℃. When catechol was used as the substrate, the measured Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) of PPO in 2nd instar larva were 3.10 mmol/min and 476.19 mmol/L, respectively; but those in pupa were 0.63 mmol/min and 50.25mmol/L, respectively, indicating that the catalytic activity of PPO in 2nd instar larva to the substrate catechol was higher than that in pupa. When L-DOPA was used as the substrate, the measured K_m and V_{max} of PPO in the 3rd instar larva were 0.49 mmol/min and 188.68 mmol/L, respectively; in contrast, the K_m (0.25 mmol/min) and V_{max} (21.79 mmol/L) of PPO in pupa were relatively lower. 【Conclusion】 Our results indicate that the properties of PPO in *B. tau* at different temperature and pH values are closely associated with its developmental stage.

Key words: *Bactrocera tau*; developmental stage; polyphenol oxidase; enzyme activity; kinetic property

1 INTRODUCTION

Polyphenol oxidases (PPO), also known as tyrosinase in mammalian (Sugumaran, 2002), are a group of copper-proteins widely distributed over the whole phylogenetic scale from bacteria to mammals (Mayer, 1987). Commonly, PPO catalyzes two distinct reactions. The first reaction is the hydroxylation of monophenols to o-diphenols (monophenolase activity) and the second is the oxidation of o-diphenols to o-quinones (diphenolase activity) which in turn are polymerized to brown, red or black pigments through a series of enzymatic and non-enzymatic reactions (Garcia-Carmona *et al.*, 1988; Hernández-Romero *et al.*, 2005; Erat *et al.*, 2010).

In insects, PPO is a key modulation enzyme during the growth and development, especially in the molting process, and is responsible for the sclerotization of their exoskeleton, epidermis pigmentation, wound healing and parasite encapsulation (Kramer and Hopkins, 1987; Wang *et al.*, 2004b; Christensen *et al.*, 2005). Because of its significance in the darkening and hardening of cuticle, PPO has received much attention from researchers in the fields of insect physiology and biochemistry. Studies on characterization of kinetic properties of PPO have been focused on many agricultural insects, such as *Sarcophage bullata* (Chase *et al.*, 2000), *Semiothisa cinerearia* (Liu *et al.*, 2004), *Plutella xylostella* (Wang *et al.*, 2004a), and *Pieris rapae* (Xue *et al.*, 2005).

Bactrocera tau (Walker) is a vegetable pest and is listed as a quarantine insect in many countries. This pest occurs in tropical and subtropical regions including China, Japan, Thailand, Malaysia, Laos, Philippines, Cambodia,

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India, Bhutan, Indonesia, and Sri Lanka (Drew and Romig, 1997; Baimai *et al.*, 2000; Drew and Raghu, 2002). It mainly infests cucumber, pumpkin, towel gourd, white gourd, and bitter gourd, as well as other fruits and vegetables, and has caused enormous economic losses for fruit and vegetable growers (Allwood *et al.*, 1999; Liu *et al.*, 2001; Chinajariyawong *et al.*, 2003). In order to prevent the damage, insecticides such as triazophos (Reddy, 1997) and pyrethroids (Borah, 1997) have been used in cover sprays on cucurbit crops. However, the pesticide remnant has caused serious damage to the environment and harm to the human health. Therefore, it is of very important significance to develop novel chemical control agents especially some inhibitors with species-specific insecticides such as prophenoloxidase inhibitors. Recently, prophenoloxidase inhibitors including with stilbene-type oxyresveratrol and tannic acid have been discovered as depigmenting agents for the prevention of dermal melanogenesis in some insects (Solano *et al.*, 2006), but no information is available on prophenoloxidase inhibitors from fruit flies.

In the present study, we examined the activities of PPO from *B. tau* at different developmental stages under various pH and temperatures, and analyzed the kinetic parameters of PPO reaction. This information will be useful in finding alternative insect control agents involved in the formation of insect epidermis and in developing prophenoloxidase inhibitors for fruit flies.

2 MATERIALS AND METHODS

2.1 Insect samples

Damaged gourds, mostly of the genus *Cucumis*, and some species of the genus *Luffa*, *Benincasa*, *Momordica* by *B. tau*, were collected from the field in June 2006 in Chongqing, China. Eggs and larvae in the damaged gourds were maintained in a rearing box under natural light conditions. A mixture of 300 – 500 female adults (20 d) from the above rearing box was transferred to a growth chamber containing fresh cucumber *Cucumis sativus* Linn that were similar in weight (50 – 100 g per gourd) to lay eggs. Two days later, these gourds with eggs were maintained in the growth chamber at 28℃, RH 80% ± 5% and photoperiod of 14L∶10D. Larvae from the above gourds were reared on the same diets, and 5 – 8 cm depth soil layer was added to provide a pupation site for the mature larvae exiting these damaged gourds until the eclosion of adults. During the course of rearing, the 1st instar larvae (1 d), the 2nd instar larvae (4 d), the 3rd instar larvae (7 d), 2 day-old pupae and 2 day-old adults were collected and then frozen at −20℃ immediately.

2.2 Enzyme preparation

Extraction of the PPO enzymes from *B. tau* was carried out according to the method described by Sánches-Ferrer *et al.* (1990) with some modifications. Samples of *B. tau* for each experiment were initially washed in cooled ddH_2O to eliminate surface contamination, and then homogenized in ice-cold sodium phosphate buffer (0.2 mol/L, pH 6.8). The ratio of the sample to sodium phosphate buffer was 1∶10 (g/mL). The crude homogenate was incubated for 30 min at 4℃ and then centrifuged at 8 000 r/min for 30 min at 4℃. The supernatant was collected and stored at −20℃. Before being assayed, the supernatant was diluted 10 times with the above buffer.

2.3 Protein estimation

The total protein content in supernatants was determined by the modified Bradford method (Bradford, 1976) using a Tecan Sunrise Microplate Reader (Tecan, Austria). The incubation mixture consisted of 0.8 mL of phosphate buffer (0.2 mol/L, pH 6.8), 5.0 mL of Coomassie blue solution G-250 (0.0025%) and 0.2 mL of enzyme solution. A total of 0.3 mL of the incubation mixture was transferred to the well of a 96-well microtiter plate, and then the absorbance at 595 nm was read at 25℃ against a blank lacking protein and compared to a bovine serum albumin (BSA) standard curve. The experiment was replicated 3 times using fresh supernatants each time.

2.4 PPO activity assay

The PPO activity was assayed spectrophotometrically as described by Benjamin and Montgomery (1973) using 4-methyl catechol as substrate. The assay mixture containing 1 mL of 0.2 mol/L sodium phosphate buffer (pH 6.8) and 1 mL of 0.01 mol/L catechol was incubated at 37℃ for 5 min and finally 0.2 mL of the enzyme extract was added to the cuvette. A total of 0.3 mL of the reaction mixture was transferred to the well and changes in the absorbance at 415 nm were measured every 30 s up to 4 min depending on the reaction rate using the Microplate Reader. The blank sample contained only 0.3 mL of substrate solution. All assays were performed in triplicate using fresh supernatants each time. One unit of the enzymatic activity was defined as a change in the absorbance value of 0.001 per min per mg protein.

2.5 Effect of pH on PPO activity

The reaction pH was adjusted from 5.0 to 8.0

by using 0.2 mol/L acetate buffer (pH 5.0 – 5.5), and sodium phosphate buffer (pH 6.0 – 8.0). The enzyme solution was pre-incubated in sodium phosphate buffer with different pH values for 1 h at 37℃. Subsequently, PPO activity was measured by using 0.2 mol/L sodium phosphate buffers at different pH at 37℃, and the assay procedure is described in 2.4. All determinations were conducted 3 times using fresh supernatants each time.

2.6 Effect of temperature on PPO activity

The enzyme solution in 0.2 mol/L phosphate buffer at pH 6.8 was incubated at various temperatures (20℃, 27℃, 34℃, 37℃, 40℃, 45℃ and 50℃) for 1 h, and then rapidly cooled in an ice bath for 5 min. PPO activities of the above solutions were assayed using 0.01 mol/L catechol as the substrate, and the assay procedure is described as in 2.4. The experiment was replicated thrice using fresh supernatants each time.

2.7 Kinetic analysis of PPO

Using catechol as the substrate, initial reaction rates of PPO activity were determined at seven different substrate concentrations (0.3125, 0.625, 1.25, 2.5, 5, 10 to 20 mmol/mL). The reaction was measured at 415 nm by the Microplate Reader and the Lineweaver-Burk plots were generated by plotting the reaction rates against substrate concentration (Chen and Kubo, 2002). Maximum velocity (V_{max}) and Michaelis-Menten constant (K_m) were determined from Lineweaver-Burk plot. At the same time, using L-DOPA as the substrate, the reaction was carried out at different substrate concentrations (0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 mmol/L) and the absorbance changes were measured at 475 nm (Wititsuwannakul *et al.*, 2002). Other procedures were the same as those using catechol as the substrate.

2.8 Data statistics and analysis

Statistical analysis was carried out with SPSS (version 12.0). Significant differences in the protein contents during various developmental stages and the PPO activities at different pH and temperatures were determined using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests at the significant level of 0.05.

3 RESULTS

3.1 PPO activity

The measured PPO activities from different developmental stages of *B. tau* were illustrated in Fig. 1. The PPO activities in the 1st, 2nd and 3rd instar larva, pupa and adult were 257.82 ± 6.36, 364.21 ± 8.21, 434.42 ± 10.41, 231.05 ± 6.39 and 358.59 ± 7.56 U/mg, respectively. As can be seen, the PPO activity continued to increase during the larval stage, and decreased dramatically in the pupal stage followed by a slight increase in the adult stage.

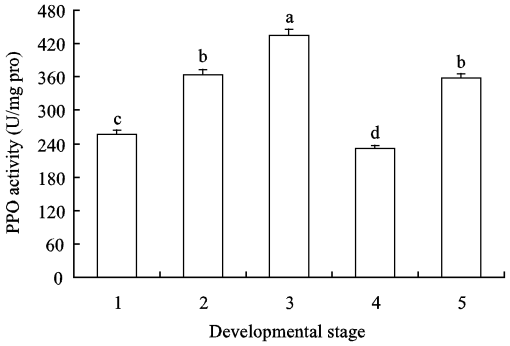

Fig. 1 The PPO activity of *Bactrocera tau* at different developmental stages

1: 1st instar larva; 2: 2nd instar larva; 3: 3rd instar larva; 4: Pupa; 5: Adult. Bars with different letters are significantly different (One-way ANOVA, $P>0.05$).

3.2 Effect of pH on PPO activity

The changes of the PPO activities at different pH ranging from 5.0 to 8.0 are shown in Fig. 2. This enzyme had the optimum activity at pH 6.5, and its activities decreased drastically at pH levels above 7.0 and below 5.0. The effect of pH on PPO activity varied at different developmental stages. Briefly, under a specific pH, the activity of PPO in the 3rd instar larva was the highest among all developmental stages; in contrast, the PPO activities in pupa and the 1st instar larva were relatively low (Fig. 2).

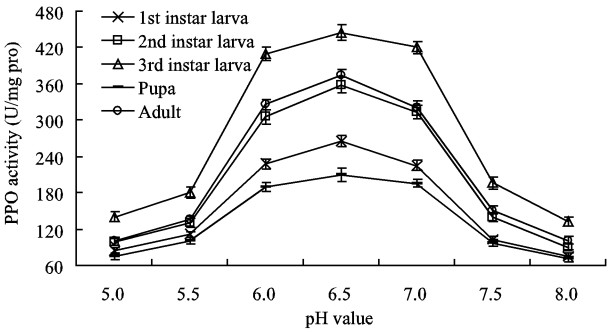

Fig. 2 Effect of pH on the PPO activity of *Bactrocera tau* at different developmental stages

3.3 Effect of temperature on PPO activity

PPO activities were measured at different temperatures varying from 20℃ to 50℃. As shown in Fig. 3, the optimum temperature of PPO reaction was around 34 – 37℃, and significant decrease in activity was noticed at above 40℃ and below 27℃. For the 3rd instar larva, the PPO activities decreased

by 62. 24% and 83. 39% at 20℃ and 50℃ respectively as compared with the initial activity. In contrast, for adult, the PPO activities decreased by 68. 67% and 86. 83% at 20℃ and 50℃, respectively.

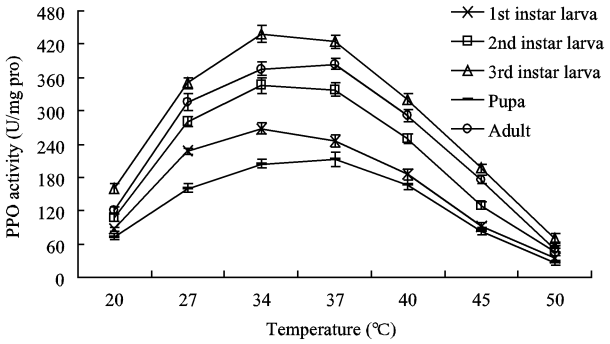

Fig. 3 Effect of temperature on the PPO activity of *Bactrocera tau* at different developmental stages

3.4 The substrate specificity and kinetic parameter

The kinetic parameters of PPO at different developmental stages of *B. tau* were determined using catechol and L-DOPA as the substrates. Fig. 4 and Fig. 5 represent the Lineweaver-Burk plots of PPO oxidation of catechol and L-DOPA at different stages of *B. tau*, and the insets show a relation between the concentration of the substrate and the reaction rate. When catechol was used as the substrate, the K_m and V_{max} values were 2. 9583 mmol/min and 208. 3333 mmol/L for the 1st instar larva, 3. 0952 mmol/min and 476. 1905 mmol/L for the 2nd instar larva, 2. 2758 mmol/min and 344. 8276 mmol/L for the 3rd instar larva, 0. 6332 mmol/min and 50. 2513 mmol/L for pupa, and 2. 3158 mmol/min and 263. 1579 mmol/L for adult, respectively. It indicated that the catalytic activity of PPO was the highest in the 2nd instar larva. When L-DOPA was used as the substrate, the K_m for adult and V_{max} for the 3rd instar larva were higher than those for other stages, indicating that the PPO catalytic activities for the 3rd instar larva and adult reach the highest levels during the insect development (Table 1).

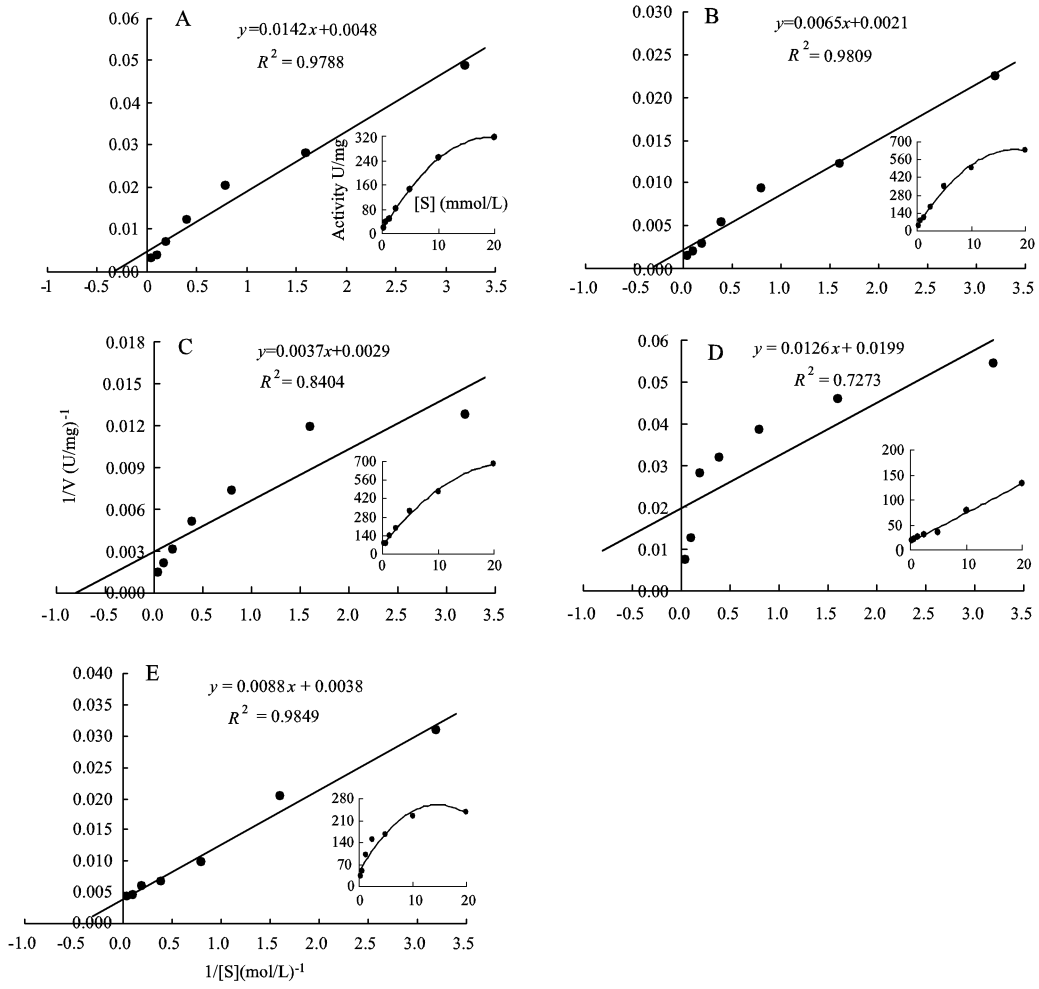

Fig. 4 The Lineweaver-Burk plots of PPO from *Bactrocera tau* at different stages for the oxidation of catechol

A: 1st instar larva; B: 2nd instar larva; C: 3rd instar larva; D: Pupa; E: Adult.

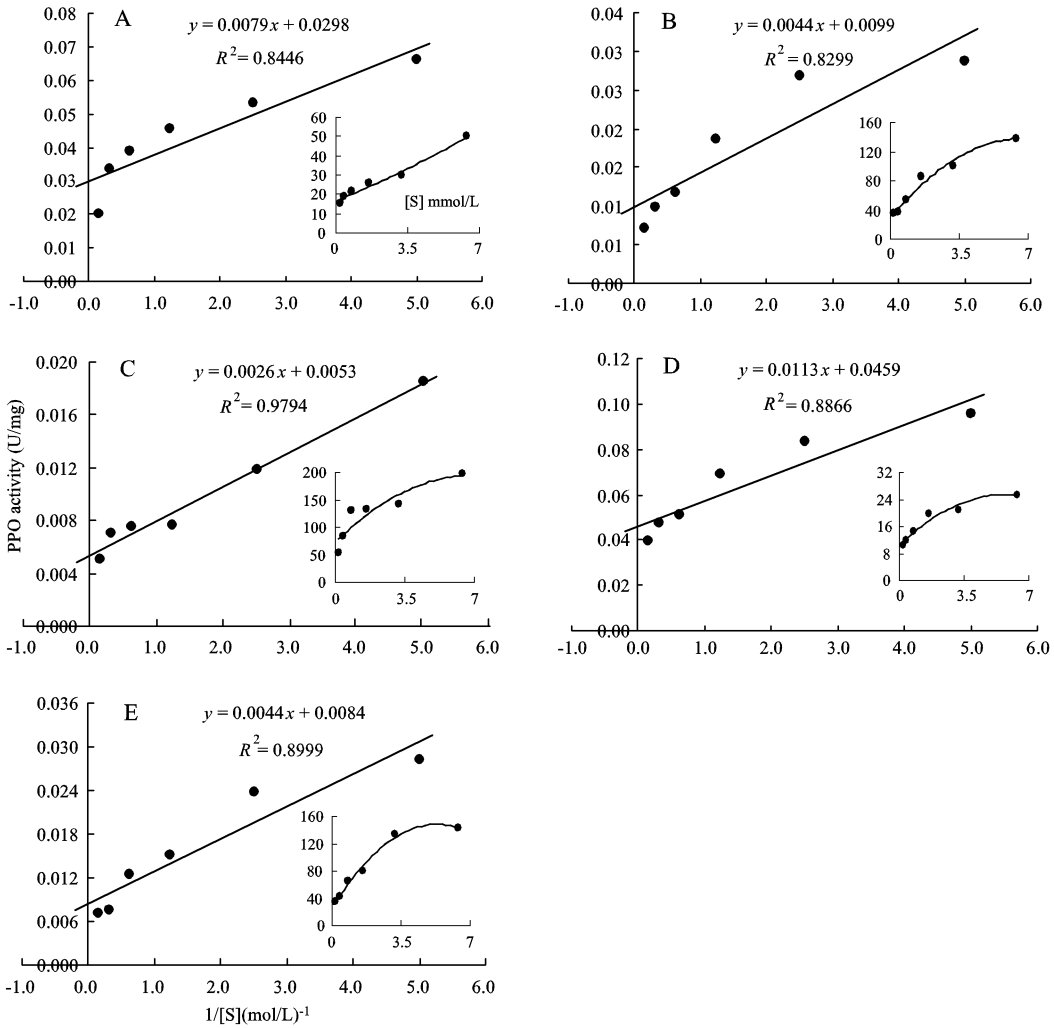

Fig. 5 The Lineweaver-Burk plots of PPO from *Bactrocera tau* at different stages for the oxidation of L-DOPA
A: 1st instar larva; B: 2nd instar larva; C: 3rd instar larva; D: Pupa; E: Adult.

Table 1 The K_m and V_{max} of PPO at different developmental stages of *Bactrocera tau* based on the oxidation of catechol and L-DOPA

Developmental stage	Catechol			L-DOPA		
	K_m (mmol/min)	V_{max} (mmol/L)	V_{max}/K_m	K_m (mmol/min)	V_{max} (mmol/L)	V_{max}/K_m
1st instar larva	2.9583	208.3333	70.4233	0.2651	33.5570	126.5820
2nd instar larva	3.0952	476.1905	153.8480	0.4444	101.0101	227.2950
3rd instar larva	2.2758	344.8276	151.5190	0.4906	188.6792	384.5890
Pupa	0.6332	50.2513	79.3609	0.2462	21.7865	88.4911
Adult	2.3158	263.1579	113.6360	0.5238	119.0476	227.2770

4 DISCUSSION

The inactive prophenoloxidase in insects was stored in the hemolymph and cuticle, and activated by some active substances at special developmental stages, especially in the molting process (Chase *et al.*, 2000; Wang, 2001). These active substances mainly contain secretions of the salivary gland, some certain lipids and proteins in cuticles. In the present study, we determined the PPO activities from *B. tau* during the whole larval, pupal and adult stages.

PPO can catalyze the oxidation of certain phenolic substrates to o-quinones in the presence of molecular oxygen (Fraignier *et al.*, 1995; Kouakou *et al.*, 2009). O-quinones formed by the PPO oxidative reaction are the initial products responsible for the hardening and darkening of the cuticle of insects. Generally, the PPO activity in insect hemolymph increases rapidly during metamorphosis.

The phenomenon was most probably due to the activators in the cuticle induced by molting hormone. This study showed that the PPO activity gradually increased during the larval stage, followed by a remarkable decrease during pupal period. To our knowledge, this is the first report of PPO activity from *B. tau* at different developmental stages. These results resemble those reported by Xue *et al.* (2004), who found that *P. rapae* larvae have higher PPO activity than their pupae and adult counterparts. However, Shi *et al.* (2000) and Li *et al.* (1992) reported that the PPO activity of *Anopheles stephensi* and *Aedes aegypti* decreased with increasing instar number. Thus, the changes of PPO activities during different developmental stages may vary among different species.

The optimum pH for PPO activity was found to vary in term of the enzyme sources and substrates (Aylward and Haisman, 1969). In general, most insects showed the maximum activities at near neutral pH values (Zhou and Feng, 1991). Our results demonstrated that PPO from *B. tau* had the optimum pH of 6.5 when using catechol as the substrate. It should be noted that Xue *et al.* (2004) reported an optimum pH of 7.0 for *P. rapae* with the same substrate. In addition, the PPO from larva showed the highest activity at 34℃ and that from adult showed the highest activity at 37℃. This suggests that the optimum temperature for the PPO activity shows a certain increase during the transition from larva to pupa and adult. The outdoor fruit fly aestivates and hibernates as pupae and adults in relatively warm and dry places, an adverse situation that might be related to the active expression of PPO.

The results presented in this work showed that there were important differences between the affinity of PPO to the substrate catechol and that to L-DOPA. When using catechol as the substrate, we found the K_m and V_{max} values were higher, but the V_{max}/K_m ratio was lower than those when using L-DOPA as the substrate. It indicated that the catalytic function of PPO to catechol was stronger, but the affinity to catechol was weaker as compared to L-DOPA. Additionally, the K_m, V_{max} and V_{max}/K_m were observed to be lower during the pupal stage compared to the other stages, most probably due to weaker metabolic activity.

We also observed that the body color of this fruit fly showed a large change from larva to adult. Generally, larvae are yellow-white, yellow and dark yellow from primary hatching to full grown larvae, then transform into brown or dark pupae and adults. Erat *et al.* (2010) revealed that the reactive o-quinones formed by the catalytic function of PPO led to the formation of yellow, brown or black pigments by cross-linking structural proteins and chitins during the discoloration of cuticle in insects. Therefore, the change of body color is probably associated with the altered PPO activities at different developmental stages of this fruit fly.

References

Allwood AJ, Chinajariyawong A, Kritsaneepaiboon SK, Drew RAI, Hamacek EL, Hancock DL, Hengsawad C, Jipanin JC, Jirasurat M, Krong CK, Leong CTS, Vijaysegran S, 1999. Host plant recprds for fruit flies (Diptera: Tephritidae) in Southeast Asia. *Raffles Bulletin of Zoology*, 7(Suppl.): 1-92.

Aylward F, Haisman DR, 1969. Oxidation systems in fruits and vegetables: their relation to the quality of preserved products. *Advanced Food Research*, 17: 1-8.

Baimai V, Phinchongsakuldit J, Sumrandee C, 2000. Cytological evidence for a complex of species within the taxon *Bactrocera tau* (Diptera: Tephritidae) in Thailand. *Biological Journal of the Linnean Society*, 69: 399-409.

Benjamin ND, Montgomery MW, 1973. Polyphenol oxidase of royal ann cherries: purification and characterization. *Journal of Food Science*, 38: 799-806.

Borah RK, 1997. Effect of insect incidence in cucumber (*Cucumis sativus*) in hill zone of Assam. *Indian Journal Agriculture Science*, 67(8): 332-333.

Bradford MM, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry*, 72: 248-254.

Chase MR, Raina K, Bruno J, Sugumaran M. 2000. Purification, characterization and molecular cloning of prophenoloxidase from *Sarcophage bullata*. *Insect Biochemistry and Molecular Biology*, 30: 953-967.

Chen QX, Kubo I, 2002. Kinetics of mushroom tyrosinase inhibition by quercetin. *Journal of Agricultural and Food Chemistry*, 50(14): 4108-4112.

Chinajariyawong A, Kritsaneepaiboon S, Drew RAI, 2003. Efficacy of protein bait sprays in controlling fruit flies (Diptera: Tephrididae) infesting angled luffa and bitter gourd in Thailand. *The Raffles Bulletin of Zoology*, 51(1): 7-15.

Christensen BM, Li JY, Chen CC, Nappi AJ, 2005. Melanization immune responses in mosquito vectors. *Trends in Parasitology*, 21: 192-199.

Drew RAI, Rachu S, 2002. The fruit fly fauna (Diptera: Tephritidae: Dacinae) of the rainforest habitat of the Western Ghats, India. *The Raffles Bulletin of Zoology*, 50(2): 327-352.

Drew RAI, Romig MC, 1997. Overview-Tephritidae in the Pacific and Southeast Asia. In: Allwood AJ, Drew RAI eds. Management of Fruit Flies in the Pacific. Australian Centre for International Agricultural Research Proceeding, Canberra. 76: 46-53.

Erat M, Şahin YN, Aksoy G, Demirkol A, 2010. Partial characterization of polyphenoloxidase from a hybridized wheat (*Triticum aestivum* L.). *European Food Research and Technology*, 231: 899-905.

Fraignier MP, Marquès L, Fleuriet A, Macheix JJ, 1995. from different fruits of *Prunus*. *Journal of Agricultural and Food Chemistry*, 43(9): 2375-2380.

Garcia-Carmona F, Valero E, Cabanes J, 1988. Effects of L-proline on mushroom tyrosinase. *Phytochemistry*, 27: 1961-1964.

Hernández-Romero D, Solano F, Sanchez-Amat A, 2005. Polyphenol oxidase activity expression in *Ralstonia solanacearum*. *Applied and Environmental Microbiology*, 71(11): 6808-6815.

Kouakou TH, Kouadio YJ, Kouamé P, Waffo-Téguo P, Décendit A, Mérillon JM, 2009. Purification and biochemical characterization of polyphenol oxidases from embryogenic and nonembryogenic cotton

(*Gossypium hursutum* L.) cells. *Applied Biochemistry and Biotechnology*, 158(2): 285 - 301.

Kramer KJ, Hopkins TL, 1987. Tyrosinase metabolism for insect cuticle tanning. *Archives of Insect Biochemistry and Physiology*, 6: 279 - 301.

Li J, Tracy JW, Christensen BM, 1992. Relationship of the hemolymph phenoloxidase and mosquito age in *Aedes aegypti*. *Journal of Invertebrate Pathology*, 60: 188 - 191.

Liu CY, Luo WC, Li FZ, Wang XY, 2004. Purification and characterization of polyphenol oxidase from *Semiothisa cinerearia* Bremer *et* Grey (Lepidoptera: Geometridae). *Acta Entomologica Sinica*, 47(2): 184 - 188. [刘春英, 罗万春, 李方正, 王晓云, 2004. 槐尺蠖多酚氧化酶的纯化及酶学特征. 昆虫学报, 47(2): 184 - 188]

Liu YZ, Lin MY, 2001. The development, longevity, fecundity and population parameters of *Bactrocera tau* (Diptera: Tephritidae) on various host fruits and artificial diet, and the female's oviposition behavior. *Formosan Entomology*, 21: 221 - 236. [刘玉章, 林明莹, 2001. 南瓜实蝇(双翅目: 实蝇科)的形态、发育、寿命及其交尾行为. 台湾昆虫, 21: 221 - 236]

Mayer AM, 1987. Polyphenol oxidases in plants - recent progress. *Phytochemistry*, 26: 11 - 20.

Reddy AV, 1997. Evaluation of certain new insecticides against cucurbit fruit-fly (*Dacus cucurbitae* Coq.) on bitter gourd. *Annals Agricultural Research*, 18(2): 252 - 254.

Sánches-Ferrer A, Villaba J, Garcia-Carmona G, 1990. Partial purification of a thylakoid-bound enzyme using temperature-induced phase partitioning. *Analytical Biochemistry*, 2: 248 - 254.

Shi CM, Huang FS, Kuang MS, Duan JH, 2000. Relationship between hemolymph phenol oxidase and melanization of oocysts of *Plasmodium yoelii* in *Anopheles stephensi*. *Chinese Journal of Parasitology and Parasitic Diseases*, 18(1): 11 - 13. [时超美, 黄复生, 况明生, 段建华, 2000. 斯氏按蚊血淋巴酚氧化酶与约氏疟原虫卵囊黑化的关系. 中国寄生虫学与寄生虫病杂志, 18(1): 11 - 13]

Solano F, Briganti S, Picardo M, Ghanem G, 2006. Hypopigmenting agents: an updated review on biological, chemical and clinical aspects. *Pigment Cell Research*, 19: 550 - 571.

Sugumaran M, 2002. Comparative biochemistry of eumelanogenesis and the protective roles of phenoloxidase and melanin in insects. *Pigment Cell Research*, 15: 2 - 9.

Wang Q, Chen X, Huang XH, Ke LN, Shi Y, Wang J, 2004a. Enzymatic characterization and functional groups of polyphenol oxidase from the pupae of blowfly (*Sarcophaga bullata*). *Biochemistry*, 69(8): 918 - 920.

Wang SD, Luo WC, Gao XX, Ding Q, 2004b. Inhibitory effects of kojic acid on phenoloxidase of diamondback moth *Plutella xylostella*. *Scientia Agricultura Sinica*, 37(9): 1316 - 1321. [王树栋, 罗万春, 高兴祥, 丁琦, 2004. 曲酸对小菜蛾酚氧化酶抑制作用的研究. 中国农业科学, 37(9): 1316 - 1321]

Wang YC, 2001. Insect Biochemistry. China Agriculture Press, Beijing. 129 - 130. [王荫长, 2001. 昆虫生物化学. 中国农业出版社. 北京. 129 - 130]

Wititsuwannakul D, Chareonthiphakorn N, Pace M, Wititsuwannakul R, 2002. Polyphenol oxidases from latex of *Hevea brasiliensis*: purification and characterization. *Phytochemistry*, 61: 115 - 121.

Xue CB, Chen QX, Wang Q, Ke LN, Luo WC, 2004. Comparison on properties of polyphenoloxidase from *Pieris rapae* (L.) in different stages and instars. *Acta Entomologica Sinica*, 47(3): 305 - 309. [薛超彬, 陈清西, 王勤, 柯莉娜, 罗万春, 2004. 菜青虫不同虫态及虫龄的多酚氧化酶性质比较. 昆虫学报, 47(3): 305 - 309]

Xue CB, Wang Q, Ke LN, Chen QX, Luo WC, 2005. Inhibitory effect of cupferron on the activity of polyphenoloxidase from *Pieris rapae* (L.) (Lepidoptera: Pieridae). *Acta Entomologica Sinica*, 48(2): 290 - 294. [薛超彬, 王勤, 柯莉娜, 陈清西, 罗万春, 2005. 铜铁试剂对菜青虫多酚氧化酶的抑制作用. 昆虫学报, 48(2): 290 - 294]

Zhou HW, Feng X, 1991. Polyphenol oxidase from Yali pears (*Pyrus bretschneideri*). *Journal of the Science of Food and Agriculture*, 57(3): 307 - 313.

南亚果实蝇多酚氧化酶的性质研究

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摘要: 【目的】为揭示南亚果实蝇 *Bactrocera tau* (Walker)不同发育阶段体内多酚氧化酶的活性与性质。【方法】以邻苯二酚为底物, 在 415 nm 波长下测定了南亚果实蝇 1, 2 和 3 龄幼虫、蛹以及成虫多酚氧化酶的活性和动力学参数。【结果】南亚果实蝇在不同发育阶段, 多酚氧化酶的活性存在明显差异, 通常 3 龄幼虫中活性最高, 为 434.42 U/mg; 蛹中最低, 为 231.05 U/mg。在 pH 6.5 时, 南亚果实蝇不同发育阶段多酚氧化酶的活性分别为 265.42, 358.34, 444.42, 210.02 和 373.99 U/mg, 但当 pH 值高于 7.0 或低于 5.0 时, 多酚氧化酶的活性则明显下降。在温度为 34℃和 37℃时, 南亚果实蝇各发育阶段多酚氧化酶的活性均较高, 当温度高于 40℃或低于 27℃时, 活性则明显下降。以邻苯二酚为底物, 2 龄幼虫中多酚氧化酶的 K_m 值(3.10 mmol/min)和 V_{max}(476.19 mmol/L)较大, 说明多酚氧化酶对底物邻苯二酚催化能力强; 蛹中多酚氧化酶的 K_m(0.63 mmol/min)和 V_{max}(50.25 mmol/L)较小, 说明多酚氧化酶对底物的亲和力和催化能力弱。当以 L-DOPA 为底物时, 3 龄幼虫中多酚氧化酶的 K_m 值和 V_{max} 较大, 分别为 0.49 mmol/min 和 188.68 mmol/L; 蛹中多酚氧化酶的 K_m 值和 V_{max} 较小, 分别为 0.25 mmol/min 和 21.79 mmol/L。【结论】南亚果实蝇体内多酚氧化酶在不同温度和 pH 值下的活性和动力学参数与虫体发育阶段密切相关。

关键词: 南亚果实蝇; 发育阶段; 多酚氧化酶; 酶活性; 动力学特性

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